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Product Release Note

LIFA System for Fluorescence Lifetime Imaging Microscopy (FLIM)

INTRODUCTION

Quantitative fluorescence microscopy uses the intensity of the fluorescence to extract information about the local concentrations of molecules that are labeled with fluorescent probes. This technique suffers from the fact that the fluorescence of the probe is permanently destructed by light-induced conversion of the probe material to a non-fluorescent compound. This photochemical process is called "bleaching" and makes it necessary to regulate the excitation dose in an economical way. Another physical property of fluorescent molecules is the fluorescence lifetime. The fluorescence lifetime is the decay time of the emission after the excitation has been stopped. The fluorescence lifetime depends on the local concentrations of certain molecules or ions. Changes in fluorescence efficiency as caused by bleaching are not accompanied by changes in fluorescence lifetime. Fluorescence lifetime imaging microscopy (FLIM) merges the information of the spatial distribution of the probe with the lifetime to enhance the reliability of the concentration measurements.

Additionally, FLIM enables the discrimination of fluorescence coming from different dyes, including autofluorescent materials, that exhibit similar absorption and emission properties but showing a difference in fluorescence lifetime.

More recently FLIM is used in combination with FRET (Fluorescence Resonance Energy Transfer) using GFP, CFP, YFP and related fluorescent proteins fused in constructs to study macro molecular interactions. The Lambert Instruments FLIM system is shown in figure 1.

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FLIM

Upon excitation fluorescent molecules exhibit delayed emission. The integrated emission of a large number of molecules shows an exponential time-coarse. In FLIM, the time constants in each pixel of the image are determined from the decay of the emission.

As an alternative to the time domain method, the LIFA fluorescence lifetime imaging system uses the frequency domain method to measure the lifetime[1]. This method uses a homodyne detection scheme and requires a modulated light source and a modulated detector. The excitation light is modulated in a sinusoidal fashion. The fluorescence intensity shows a delay or phase-shift with respect to the excitation and a smaller modulation-depth. The phase-shift and modulation-depth depend on the decay constants of the fluorescent material and the modulation frequency. It can be shown that the lifetime of a fluorescent material can be determined from the phase-shift by the following relation:

$$\tau_\vartheta = \frac{1}{\omega}\tan(\vartheta)$$

where ω is the angular frequency of the modulation and ϑ is the phase-shift. Equally the lifetime can be determined from the modulation depth m which is the relative modulation depth of the emission signal as compared to the excitation, by:

$$\tau_m = \frac{1}{\omega}\sqrt{\frac{1}{m^2} - 1}$$

In case of a mono-exponential material, the lifetime derived from the phase should be equal to the lifetime derived from the modulation depth.

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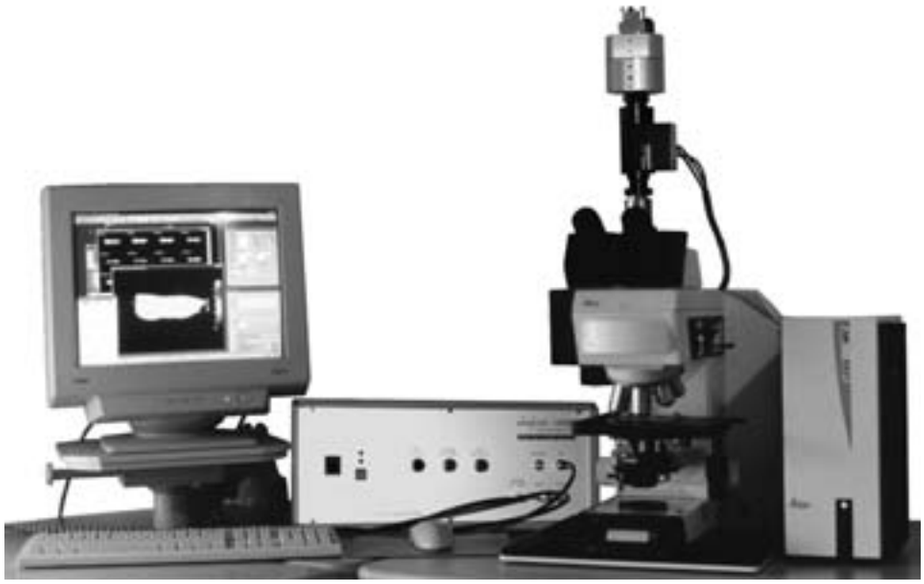


Fig. 1. The Lambert Instruments FLIM system

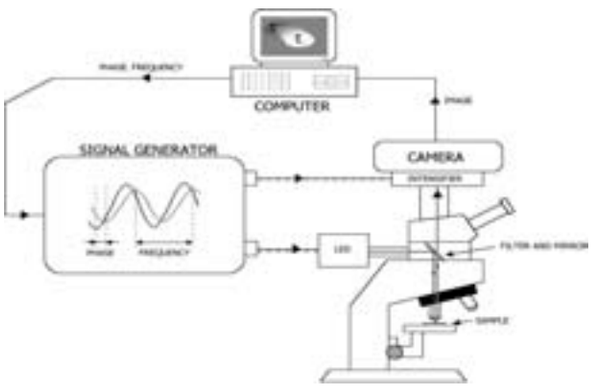


Fig. 2. FLIM system setup: Wide field fluorescence microscope and LIFA attachment for FLIM.

For a more detailed background about FLIM methods, experiments and results see the literature[2].

SYSTEM COMPONENTS

The FLIM system uses a standard fluorescence microscope. The complete setup of the Lambert Instruments FLIM system and microscope is shown in Figure 2.

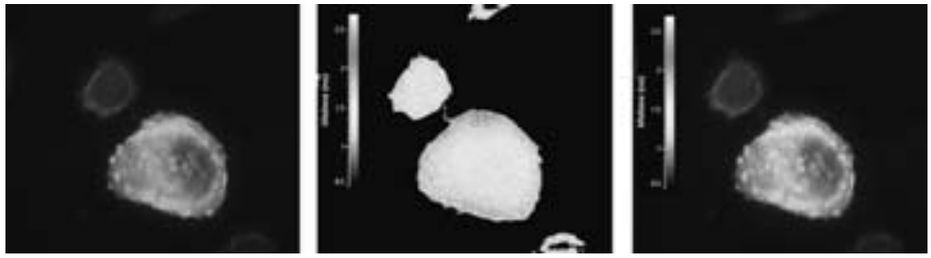
Light source

In order to develop an affordable and easy-to-use FLIM system we have looked at low cost light sources that can be directly RF modulated. Ultra bright LEDs with different wavelengths have been tested successfully. Special LED's are selected that can be modulated between 1 and 100 MHz. Thanks to their non-coherency, LED's do not cause unwanted interference patterns as lasers do. The LED is mounted in the standard arc lamphouse that is connected to the excitation light port of the microscope. It is also possible to use a modulated laser diode or a gas laser that is modulated with an AOM.

Camera/Detector

The modulated fluorescence coming from the sample and containing the lifetime information of each pixel has to be detected in order to retrieve the spatial and lifetime information. This is achieved by imaging the sample with the microscope on the input of an image intensifier that demodulates and intensifies the image. The demodulated image then is projected on the CCD image sensor of a camera. This is done by either using relay lenses or via a tapered fiber-optical image guide

The Lambert Instruments LIFA system uses a modulated 18 mm Gen II proximity focused image intensi-



Intensity Lifetime Lifetime + Intensity

Fig. 3.

fier. The intensifier is mounted with the relay lens in a separate housing and can be connected via C-mount to the camera port of the microscope. The output of this unit is connected via C-mount to the CCD camera. The selected camera uses a progressively scanned 2/3 inch interline CCD having 1.3 million pixels. The 12 bit image data are transferred to the computer via an IEEE-1394 interface.

The image intensifier gain is modulated at the same frequency (1-100 MHz) as the excitation of the object is modulated with. The image is demodulated by the relatively long decay time of the phosphor anode screen of the intensifier.

Signal generator

The signal generator produces two sine waves at frequencies from 1 to 100. The phase difference is selectable from 0° to 359°. One output signal is coupled to the LED. The second output signal is coupled to the image intensifier. The signal generator is remote controlled via RS-232. The control software is integrated in the FLIM software.

LIFETIME MEASUREMENTS

From reference and sample data, the lifetime image is automatically calculated and presented.

The quality of Fluorescence lifetime Imaging can be greatly improved by combining the intensity information from the monochrome fluorescence image and the calculated lifetime image. Structures that are visible in the intensity image but are masked in the lifetime image will remain visible in the combined image. Images that have been made with the LIFA system at the EMBO Practical Course “Light Microscopy of Live Specimens” at EMBL in Heidelberg (May 2002), showing MCF7 cells with ErbB.1-GFP as donor and Py72/Cy3 as acceptor. They are post processed to show the combined lifetime and intensity image.

REFERENCES

1. Patents owned by Photonic Resarch Systems Ltd.
2. T. W. J. Gadella, “Fluorescence Lifetime Imaging Microscopy (FLIM)”, *Microscopy and analysis*, pp. 13–15, May 1997.